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ISOLATION AND CHARACTERIZATION OF ERYTHROCYTE AND PARASITE MEMB--ETC(U)
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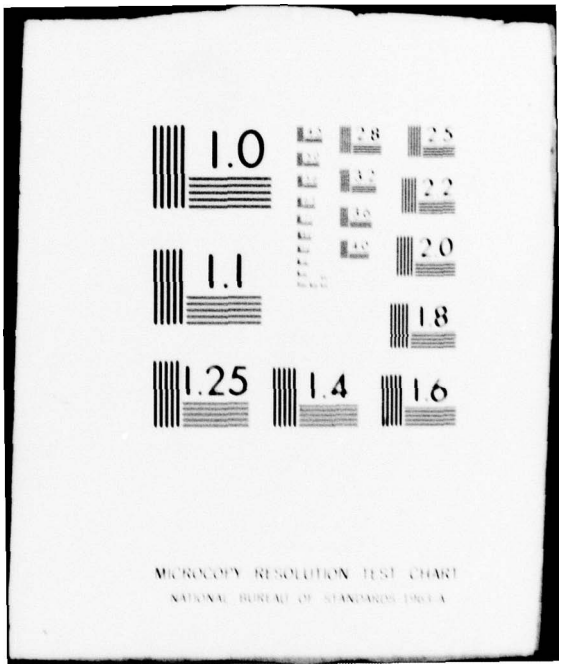
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LEVEL II

Isolation and Characterization of Erythrocyte and Parasite Membranes from Rhesus Red Cells Infected with P. Knowlesi

III

Annual Summary Report

June 1, 1976 - May 31, 1977

Donald F. H. Wallach

April 1978

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Pl. knowlesi, erythrocyte-membranes, surface-radioiodination, nitrogen-decompression, differential-centrifugation, dodecyl-sulfate-polyacrylamide-gel-electrophoresis.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Pure erythrocytes were isolated from normal rhesus monkeys (Macaca mulatta) and from animals infected with Plasmodium knowlesi. The external surfaces of both normal and parasitized cells were ^{125}I-labeled by lactoperoxidase-catalyzed iodination. The erythrocyte membranes were vesiculated in the cold and at physiological ionic strength by nitrogen decompression; 600 psi were used for normal erythrocytes and 280-290 psi for parasitized cells. Host cell membrane and intact, released parasites were isolated by differential centrifugation

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20. Abstract (continued)

and Ficoll-Hypaque density gradient centrifugation; both the yield and degree of purity were good. The fractionations were monitored by (a) microscopy, (b) distribution of erythrocyte membrane-bound ^{125}I, and (c) analytical dodecyl sulfate polyacrylamide gel electrophoresis. Major differences observed between the membranes of normal and parasitized cells included: (a) presence of an apparently new iodinatable glycoprotein component (Mol wt ~125,000 - 130,000), (b) apparent monomerization of a glycoprotein normally of Mol wt ~ 98,000, (c) apparent deletion of an iodinatable protein (Mol wt ~ 52, 000), and (d) possible selective adsorption to membranes of a parasite protein (Mol wt 68,000).

Background and Progress Report

To understand the biochemistry and immunology of erythrocytic plasmodial forms, and to develop necessary new chemo- and immunotherapeutic measures for their control requires effective procedures for the separation of parasite and host cell material. Numerous techniques have been applied to the separation of the membranes of parasitized erythrocytes from the enclosed parasites (1-9) but efforts in this direction have been less than satisfactory until very recently. Virtually all of the experimentation in this area has focussed on methods of erythrocyte lysis that might achieve release of intact parasites, namely: osmotic shock (1,2), use of proteases (3), shear by the French press (4), sonication (5) saponin lysis (6,7) and immune lysis (8,9). Indeed, except for the study in the Progress report (10), fractionation criteria in these investigations have been primarily morphological, have not rigorously applied modern technologies for subcellular fractionation (e. g. 11-13), and have essentially neglected the fate of erythrocyte membranes, except to the extent that they constitute morphologically recognizable contaminants of parasite isolates. The latter omission is regrettable in view of the probability that the intraerythrocytic maturation of malaria parasites brings with it not only morphologic (14, 15) biochemical (15) and functional (e. g. 16-18) modifications of the host cell membrane, but immunological alterations as well (e. g. 19).

Abstract

1. Pure erythrocytes are isolated from normal rhesus monkeys (Macaca mulatta) and animals infected with Plasmodium knowlesi.
2. The external surfaces of both cell categories are ^{125}I-labelled by lactoperoxidase-catalyzed iodination.
3. The erythrocyte membranes are vesiculated in the cold and at physiological ionic strength by nitrogen decompression. 600 psi is used for normal erythrocytes and 280-290 psi for parasitized cells.
4. Host cell membranes and intact, released parasites are isolated in good yield and purity by differential centrifugation and Ficoll-Hypaque density gradient centrifugation.
5. The fractionations are monitored by (a) microscopy (b) distribution of erythrocyte membrane-bound ^{125}I and (c) analytical dodecyl sulfate polyacrylamide gel electrophoresis.
6. Major differences between the membranes of normal and parasitized cells are observed. These include (a) appearance of an apparently new, iodinatable glycoprotein component (Mol. wt. $\sim$ 125,000 - 130,000), (b) apparent monomerization of a glycoprotein normally of Mol. wt. $\sim$ 98,000, (c) apparent deletion of an iodinatable protein (Mol. wt. $\sim$ 52,000), (d) possible selective adsorption to membranes of a parasite protein (Mol. wt. 68,000).

Progress Report

Introduction

As part of a program to characterize the host cell membrane modifications that occur during intraerythrocytic maturation of Plasmodium knowlesi, and the different properties of parasite and host cell membranes, we have developed an effective approach for the separation of parasite and host cell components (10). An extension of this approach, as well as detailed characterization of parasite membranes and of parasite-related host cell membrane-protein modifications are the objectives of this application.

Summary of Proposed Work

1. Continuing initially with the Simian model, the technologies described in item (a) will be modified to give greater cell disruption with lesser parasite damage and better-yield separation of host cell membranes from parasites. For the latter the value of introducing dextran gradients will also be explored.

2. The labelling/monitoring procedures will be extended by techniques for *in vitro* labelling of host cell membrane sialate and the metabolic labelling of parasite components.

3. The proteins of pure host cell membranes will be further characterized by isoelectric focussing, bidimensional immune electrophoresis, bidimensional focussing - immune electrophoresis and bidimensional DS-PAGE approaches. This involves comparisons with similar analyses on host membranes from normal cells and on parasites, along the lines summarized in (a). The aims here are to (i) define parasite-induced changes in host cell membranes, and (ii) define "candidate" antigens.

4. Techniques currently in use in this laboratory on other systems will be applied to determine conditions required to maintain the permeability characteristics of isolated schizonts.

5. The schizonts isolated after metabolic labelling will be labelled at their external surfaces by lactoperoxidase-catalyzed radioiodination.

6. Conditions will be established to achieve parasite disruption by nitrogen decompression without damage to intracellular organelles.

7. Disrupted parasites will be fractionated into subcellular organelles and soluble fractions, using differential and isopycnic ultracentrifugation and quantitative monitoring using extrinsic and intrinsic markers.

8. Purified membranes will be characterized primarily by the techniques listed in (b3) above.

9. Our approach will be transferred to human *in vitro* systems when feasible.

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